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MICROBIOLOGICAL SURVEYS OF CITRUS PROCESSING PLANTS DURING THE 1948-1949 SEASON

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The importance of good sanitation in the handling and processing of citrus fruits is generally recognized throughout the industry. Effective sanitary practices are essential if high quality products are to be produced even if they are pasteurized, and the increasing production of unpasteurized frozen concentrates has made greater attention to these problems imperative. A major phase of the bacteriological and microbiological work of the Citrus Products Station has been the systematic accumulation of data which can serve as a reliable and factual basis for the improvement of sanitary conditions and the development of more effective procedures for maintaining them at the highest level.

It is felt that a complete summary of the observations made and results obtained during the 1948-1949 season will be of value although it is not to be expected that the same condition will be duplicated in future seasons. Specimens collected from juice canneries or concentrating plants for analysis were as follows:

1. Unwashed peel
2. Washed peel
3. Peel at extractors
4. Juice to finishers
5. Reconstituted juice or juice from blending tank
6. Reconstituted, canned concentrate

All specimens were packed in ice as soon as collected.

The inspection of the first three specimens revealed facts concerning the contaminated exterior of raw fruit as taken from the bin, the efficiency of the washing operation for removing the surface contamination, and the accumulation in exterior flora of the fruit as it passed along the sizer belt to the extractor.

The examination of the three remaining specimens was intended to show the contamination of the juice due to build-up on the machinery and juice lines because of nascent growth and to contamination from the fruit both interior and exterior.

The canned specimens of concentrate collected on the line following the closure machine would contain the contamination originally present in the juice previous to concentration plus that introduced

with added-back pulpy juice. The counts of micro-organisms would be affected by the breaking apart of clumps during the evaporation process and slush freezing. In some plants population numbers were reduced because of preheating and then rapidly cooling before filling the can or in some instances by moderate heating in conjunction with pressure.

Four media were employed to obtain microbiological population figures of the six items investigated for each plant. These media were selected because they were commonly used for total count determinations by the routine laboratories in this area. The media were inoculated with dilutions of the same specimen and after a period of incubation (48-72 hours at 37° C.) counts were made to obtain a comparison of the microbial population for each item examined. The media were rated according to the number of colonies produced.

When dilutions were prepared for inoculation purposes, 1 ml. of 1-10 dilution of each sample was introduced into each of four plates and mixed with E. M. B. agar after cooling to 45° C. Inoculations of 1-10 dilutions of orange juice were also made into lactose as an enrichment medium and after gas was produced following incubation, streak inoculations were made on E. M. B. solidified plates. These plates were incubated at 37° C. for 48 hours, but were observed after 24 hours. All colonies that grew on the surface of E. M. B. agar and produced a sheen were removed to agar slants, incubated, and were later purified. The cultures were subjected to the gram stain test. Gram negative cultures were inoculated into media for the "Imvic" test and later as time permitted, other physiological characteristics were determined according to standard procedure for specific identifications.

Contamination of Fruit Peel

Pieces of 1-inch stainless steel tube, 4 inches long, were sharpened on the outside edges of one end to facilitate the cutting of peel samples from fruit. Each piece of pipe was wrapped in paper individually and sterilized; thus the pieces were carried on the inspection trip without much danger of being contaminated. After removing the sampler from its sterile wrapper, the fruit to be sampled was held in one hand, care being exercised to prevent the fingers from touching areas to be removed for peel contamination tests. A determined effort was made to select fruit that was firm and sound in appearance to avoid possible infection from the interior flora of deteriorating tissue. One tube was used to cut three plugs: one each at the stem and stylar ends and one about midway between these two points. The plugs were left in the tube which was rewrapped in sterile paper and placed upright with the cutting end up. When the specimens were returned to the laboratory each tube was unwrapped carefully and the plugs were pushed into 100 ml. of sterile water in a 500 ml. flask by means of sterile metal strips so that each flask contained the peel plugs from one fruit. The contents of each

flask were agitated 25 times; the liquid was diluted and used for plate inoculations. The numbers of colonies listed in Tables I, II, III, represent numbers per plug of 4.2 sq. cm. of peel area. The data collected by using this method are not considered quantitative; to achieve quantitative results, more specimens should have had to be taken at each sampling. The results are useful, however, in that they indicated the general trend of contamination on unwashed fruit and the effectiveness of the washing operation.

Each plant does not treat its stored fruit the same. Plants indicated in the tables as E and H wash their incoming fruit before it goes to the storage bins. Usually a little residual chlorine can be detected in the wash water. They also give their storage bins a germicidal wash at irregular intervals. The bins are allowed to stand unfilled for convenient periods, depending upon the need for space. The fruit is taken from these bins, washed with detergent, and rinsed with chlorinated water high in residual chlorine. All of the remaining factories store the fruit as received without preliminary washing. The plant designated M used untreated well water for washing and rinsing the fruit, while the remaining plants in this survey used chlorinated water and detergents to cleanse the exterior of the fruit before going to the extractors. Some operators use chlorine in water at very high levels. The amount of chlorine retained as residual in the water was not always stated, although the operators of some plants talked freely of the residual chlorine and the detergent used. Such data are not complete and are not included in this report.

Table I contains the analytical data of the fruit peel samples taken from storage bins. It will be noted that in some plants the fruit carried a contamination which was low. This was usually observed when packing-house fruit of good quality was being used, especially if it had been handled promptly. Grove-run fruit always carried the organisms natural to the environment in which it was grown, but grove-run fruit or packing-house fruit that had been mishandled and held over a long period of time presented a problem to the processor; some of the very high figures in Table I are indicative of just such fruit.

Washing the fruit brought a reduction in numbers of organisms on the exterior regardless of the method employed (Table II). The analysis shows that fruit frequently picked up considerable contamination as it passed along the sizer belt to the extractors (Table III). The sizer belts in most plants get attention at cleanup time only. This cleaning schedule may include a good to fair wash-down with water under pressure each 4 hours, with a more thorough cleanup once each 24 hours; also when the plant was shut down after 72 hours of operation. In other plants special attention was given to this equipment almost constantly by having the belt travel through a germicidal solution at each revolution. Washed fruit passing over the sizing equipment when both were relatively dry gathered less contamination during its travel than if it had passed over equally unsanitary surfaces when both were damp.

Contamination in Juice from Extractors and Juice Lines to Finishers

The mechanical extractors used in the individual plants include in this survey are as follows:[1]

Plant A - Brown
B - Brown and whole fruit juice extractors
C - Citromat
D - Fauld's rotary
E - Fauld's rotary
F - Fauld's rotary
G - Fauld's rotary
H - Whole fruit juice extractor
I - Citromat
K - Fauld's rotary
L - Fauld's rotary
M - Fauld's rotary
N - Fauld's rotary

The design of extractor in use in a given plant did not insure the production of a low-count juice. One make of extractor may be more easily cleaned than another but unless the cleanup was thorough and frequent, or the interior of the fruit was relatively free from micro-organisms, the micropopulation figures for the juice were high. A study of Table IV showed the condition in the plants inspected during the season. No plant was ever found to be in a poorly cared for, sloppy, or untidy condition. Often the quality of fruit was found to be of low grade which made it practically impossible for the operator to maintain his equipment in good sanitation to produce low-count juice. Even fruit which the buyer accepted as sound may have been far past its peak of maturity or may have been held for a long time in a storage bin or in a fruit truck carrier before it was used in a processing plant. Such fruit may appear sound from the exterior and have a highly infested interior. Fruit of this description usually carried a high contamination of both yeast and bacteria. When the juice was extracted, the equipment became increasingly seeded, yeast cells particularly found suitable conditions for growth and added to the microbialpopulation of the product. Frequent cleanup periods were necessary and fruit of better quality was usually demanded.

Contamination in the Blending Tank

Juice coming out of the finisher usually had a lower count per ml. than when it entered; the removal of pulp generally reduced the numbers of organisms per ml. of juice, unless the finisher had not been cleaned properly. In general, the number of organisms had not increased

[1] The mention of trade products does not imply their endorsement by the Department of Agriculture over similar products not mentioned.

noticeably by the time the juice had reached the blending tank unless pulp had been added back. These observations cannot be substantiated by comparing Tables IV and V. If the initial yeast infestation were high and some growing time permitted, an increase in numbers was evident. Tanks without proper cooling equipment favored the increase in microbial counts and even a low-count juice would not remain so for very long if not cooled. Short holding times and low temperatures help to keep the counts low. These generalizations also apply to concentrate plants; the citrus concentrate in blending tanks may gain a high population for the same reasons stated for single strength juice, except that organisms are not apt to make a rapid early growth (show a longer lag period) in concentrate. The plate counts are listed in Table V for fresh single-strength juice as collected from blending tanks in canneries and for reconstituted juice prepared from concentrate taken from blending tanks in concentrate plants. In either case the blends had been made ready for the pasteurizer or the continuous slush freezer respectively.

Examination of Canned Concentrate

The plate counts for unpasteurized concentrate as packed in the processing plants are listed in Table VI. The sealed cans were opened aseptically, dilutions were made, and plates were inoculated according to standard procedure. The concentrate samples from plants B, C, G, I, and L had passed through the continuous slush freezer and filling machine; otherwise they were analogous to the samples listed in Table V. It may be noted that in the seven out of ten slush frozen samples there was an increase in population numbers per ml. of reconstituted juice. It is probably true that the apparent increase was due to the breaking up of clumps which in turn afforded a better distribution of cells in the inoculated plates. Other specimens gave a decrease in numbers per ml. of reconstituted juice; death or injury may have been the cause. The medium that produced the highest count in the examination of frozen concentrate (Table VI) did not rank first with the listing in Table V. Samples from plants D, E, and H were not slush frozen previous to filling the cans. Escherichia coli was not found in the samples listed in Tables V and VI.

Examination of Fresh Juice Used for Pasteurization Studies

Throughout the citrus season, single-strength fresh juice was purchased by this station at intervals from commercial canneries close to the station for the purpose of pasteurization studies. The first six tables of this report covered the examination of plants scattered over the citrus-producing area. The data in Table VII of this report involve not more than three plants with most of the juice coming from one plant and in any case restricted to the immediate Winter Haven area. The numbers indicate the yeast and bacterial population in orange or grapefruit juice at intervals from October 8, 1948 to May 1949.

Fifteen of the twenty-six juice samples were plated on the four media used in the plant survey study. In that respect they were compared to the 15 reconstituted orange juice samples listed in Table VI and cover almost the identical portion of the fruit season. The numerical rating for the media, based on averages, in Tables VI and VII are not the same. This may be accounted for in this way: Table VI reported results from several plants over a wide area based on the analyses of reconstituted orange juice. Table VII is based on both orange and grapefruit juice collected from one plant principally. Data presented in Table VII do not present the average condition of plants for citrus fruit area, but do indicate what might happen by limiting survey studies to too few plants and a restricted area. The single-strength juice was always collected from fruit extracted during the early morning. The equipment should have been clean and clumps of organisms due to accumulation and growth should have been at a minimum. In spite of that, the numbers of organisms per ml. of juice were high in several instances. These figures did not correlate with the known sanitation of the plant's equipment, but more aptly with the quality of the fruit used.

Rating of Media for Each Item Examined

The media involved in this study are those used by the control laboratories in this citrus fruit area. It was hoped that by making a survey and plating each specimen on these media, the most acceptable medium for the purpose could be found. Each laboratory might then employ a common medium for its investigations and the results from all sources could be interpreted and the product standardized on the basis of results from that one medium. One medium for use in routine plating is desirable in that the laboratories have many specimens to plate during the work period. Since no one medium was used uniformly, an acceptable interpretation of results could not be made. It would be desirable to use a medium that could be prepared easily and which would support the growth of the greatest number and kinds of organisms present in the specimen. Generally speaking, a medium with a large amount of sugar in the formula would encourage some bacteria to produce "spreader" growth as well as a rapid growth of some yeasts that could compete with the spreaders. Some bacteria and yeasts, because of slow initial growth, would not be encouraged or given a chance to appear due to crowding and over-growth. Persons in this area who were responsible for the work done in their laboratories were not in agreement on this matter; some felt that a determination of the bacterial flora was not essential when investigating plants or evaluating the prospects for quality in the finished product. They emphasized the value of knowing the yeast flora. This laboratory has consistently used dextrose tryptone agar in microbiological analysis and, in conjunction with it, such media as it was thought advisable to show special flora. Dairy control laboratories and frozen food laboratories use tryptone glucose extract agar. They have used this medium over a long period and are able to make a reasonably accurate generalization concerning a food

product after determining the plate counts. Microscope slide spot checks made coincident with the plate counts showed that these two media just mentioned supported about a 50-50 growth of yeast and bacteria with a great variety of bacteria present. Lindegrin's agar and Sabauroud's agar supported a very high percentage of yeast colonies with bacterial colonies represented in limited varieties. Lindegrin's agar supported a greater variety and number of yeasts than did Sabauroud's agar. The formulae for the media employed in this survey are found in Table X.

The rank of each medium, based entirely on numbers, is given in Table VIII for each of the six items or specimens examined from several plants in the area. Dextrose tryptone agar scored the highest rank, Lindegrin's agar second, tryptone glucose extract agar third, and Sabauroud's agar fourth. A glance at Table VII where sampling of juice was restricted to the immediate area for approximately the same period as item five in Table VIII shows the rank of media to be different. Sabauroud's agar ranked first, Lindegrin's agar second, tryptone glucose extract agar third, and dextrose tryptone agar fourth. This may be the reason for some investigators having a conscientious preference for a certain medium when the medium of their choice was selected after many samplings over a fruit season. Along with this observation, another condition might be mentioned. Refer to Table VI and note the variation in numbers reported when the product of one plant is compared with that of another for a similar period in the same season. When this and other comparisons are made using tables listing numbers of organisms from specimens collected in the process, one may note that individual plant investigators could not offer sound advice on use of laboratory media to be applied to the whole citrus fruit producing area. What they could advise may be true of their plant and products, if conclusions were drawn after many analyses had been made.

The kinds of organisms present in the plants should receive more consideration. The flora varies with the treatment and quality of the fruit during a season as well as being affected by seasonal variations. Some of the variants in flora among processing plants may be enumerated as yeasts that grow on eosin methylene blue agar and produce small colonies with a metallic sheen, bacteria that ferment orange juice, sarcinae forms, and variations in numbers and kinds of coliforms. Some of these organisms may be found in certain plants almost anytime and seldom found in others. Because of the complications involved, these observations have not been tabulated.

The statements in this report are made after reviewing the results from the analysis of collections of this season. There is no assurance that data collected next season will substantiate these statements.

Use of Buffered Dilution Water for Plating

A point of issue has arisen frequently relative to the use of buffered dilution water when preparing a citrus juice inoculum. In this Laboratory such a practice has not been considered necessary for the usual plating of citrus fruit juices prepared in dilution for inoculation purposes. Because of differences of opinion, a simple experiment was done; MacIlvaine's buffer solution was prepared as directed in Lange's Hand Book of Chemistry (1934), page 696. Orange juice and grapefruit, early season, was buffered aseptically to pH 6.0 - 6.5 and plated; controls of the same samples unbuffered were plated, each in 1 to 1,000 dilution. The results are listed in Table IX. When 1 ml. of orange juice or grapefruit juice, unbuffered and undiluted, was added to 15 ml. of melted dextrose tryptone agar, pH 7.0 - 7.4, the pH of each medium after the juice was blended thoroughly was pH 6.0 and 5.8, respectively. Juice in the early season was of high acidity and if one ml. of such juice produced no greater change in pH than that, certainly one ml. of 1-1,000 dilution per plate in 15 ml. of medium would not alter the pH to such a degree that growth would be inhibited. The plate counts were such as not to encourage one to do the extra work which the operation would involve.

Conclusions

1. Unwashed fruitpeel carried the normal contamination of the grove unless there was a build-up due to a long holding time in storage.

2. Any method used for washing fruit reduced the surface contamination but plants using detergents and chlorine do a more thorough job.

3. The sizing equipment constituted a principal source of contamination on the fruit exterior as it traveled from the washer to the extractor. The contamination was sufficiently significant to make that equipment a major point for inspection and control.

4. The quality of fruit used was of major importance when endeavoring to produce a low-count juice provided the equipment were kept clean. Fruit with clean exterior did not insure a low-count juice from the extractors.

5. The freezing of citrus fruit concentrate usually produced an apparent increase in the numbers of organisms per ml. of juice.

6. Lindegrin's agar and Sabauraud's agar produced a high percentage of yeast colonies. Lindegrin's medium proved to be the better for yeast flora determinations. Dextrose tryptone agar produced the greatest number of colonies from a given inoculum; bacteria and yeast were present in about a 50-50 ratio. Data from one plant or a restricted area were not indicative of the condition over the entire citrus producing area.

7. Standardization should not be effected on the basis of numbers only. Kinds of organisms present should receive much consideration. Supplementary media should be employed for selective and differential purposes.

8. Buffering citrus juice in preparation for plating was not proved to be a useful practice.

9. Escherichia coli was not found in these investigations.

Table I.--Unwashed peel - no. per 4.2 sq. cm. of area

Inoculation - 1 cc. of 1-1000

Plant	Media 1	2	3	4	Date
A - j	9,000	11,000	9,000		1/11/49
B - c	31,600	24,800	22,133		1/14/49
C - c	53,666	158,666	98,000	37,333	1/21/49
D - c	21,120	45,866	41,600	11,200	2/1/49
E - c	13,200	12,266	21,600	10,000	2/4/49
B - c	422,000	420,000	420,000	350,000	2/8/49
A - j	12,000	64,000	66,000	71,000	2/25/49
F - j	30,333	69,300	71,866	32,666	3/4/49
E - c	6,200	5,000	7,000	7,200	3/8/49
G - c	2 PPL	3 PPL	1 PPL	1 PPL	3/11/49
H - c	51,000	70,000	76,066	48,766	3/15/49
I - c	1,800	3,200	6,600	1,200	3/17/49
C - c	277,666	882,000	130,666	sp.	3/18/49
J - j	326,666	328,500	56,000	261,333	3/22/49
K - j	6,566	12,866	14,966	11,666	3/24/49
L - c	sp.	0	0	sp.	3/25/49
G - c	24,000	18,000	26,000	1,466	3/31/49
B - c	14,666	20,000	19,000	16,500	4/1/49
M - j	6,000	12,000	9,800	7,000	4/6/49
I - c	9 PPL	7 PPL	9 PPL	7 PPL	4/8/49
F - j	2 PPL	2,666	9 PPL	6 PPL	4/12/49
N - j	98,000	45,266	30,333	73,500	4/14/49
H - j	1 PPL	0	0	1 PPL	4/19/49
E - c	82,666	61,333	88,000	65,066	4/22/49
Total	24(1,488,249	24(2,266,729	24(1,214,030	22(1,005,896	
Average	62,010	94,447	50,585	41,912	
Rating	2	1	3	4	

Key:

j - Juice canning plant
c - concentrate plant
sp. - spreaders
0 - no growth
PPL - Number per plate, not included in average

Media:

1 - Lindegrin's agar
2 - Dextrose tryptone agar
3 - Tryptone glucose extract agar
4 - Sabauroud's agar

Table II.--Washed peel - no. per 4.2 sq. cm. of area

Inoculation - 1 cc. of 1-1000

Plant	Media 1	Media 2	Media 3	Media 4	Date
A - j	6,666	4,666	4,333		1/11/49
B - c	22,633	16,500	179,666		1/14/49
C - c	--	--	--	--	1/21/49
D - c	2,566	2,333	1,633	0	2/1/49
E - c	1 PPL	1 PPL	0	0	2/4/49
B - c	0	4 PPL	4 PPL	2 PPL	2/8/49
A - j	--	--	--	--	2/25/49
F - j	1	8,233	9,599	4,333	3/4/49
E - c	--	--	--	--	3/8/49
G - c	0	0	1 PPL	1 PPL	3/11/49
H - c	--	--	--	--	3/15/49
I - c	700	1 PPL	2 PPL	2 PPL	3/17/49
C - c	2 PPL	8 PPL	7 PPL	4 PPL	3/18/49
J - j	4,333	11,700	12,266	7,800	3/22/49
K - j	2 PPL	1 PPL	0	2 PPL	3/24/49
L - c	0	1 PPL	1 PPL	1 PPL	3/25/49
G - c	0	1 PPL	4 PPL	0	3/31/49
B - c	14,600	18,133	19,200	15,066	4/1/49
M - j	4,266	25,333	32,000	11,700	4/6/49
I - c	1 PPL	0	2 PPL	1 PPL	4/8/49
F - j	2 PPL	13,600	10,033	1,633	4/12/49
N - j	55,466	79,333	55,466	23,333	4/14/49
H - j	--	--	--	--	4/19/49
E - c	43,200	37,333	40,000	25,066	4/22/49
Total	19(154,430	19(207,164	19(364,196	17(88,931	
Average	8,128	10,903	1,921	4,681	
Rating	2	1	4	3	

Key:

j - juice canning plant
c - concentrate plant
0 - no growth
PPL - Number per plate, not included in average

Media:

1 - Lindegrin's agar
2 - Dextrose tryptone agar
3 - Tryptone glucose extract agar
4 - Sabauroud's agar

Table III.--Peel at extractor - no. per 4.2 sq. cm. of area

Inoculation - 1 cc. of 1-1000

Plant	Media 1	Media 2	Media 3	Media 4	Date
A - j	11,333	6,800	5,766		1/11/49
B - c	*	87,500	87,500		1/14/49
C - c	0	1 PPL	1 PPL	0	1/21/49
D - c	30,333	36,400	28,000	19,133	2/1/49
E - c	4 PPL	4 PPL	sp.	6 PPL	2/4/49
B - c	3,766	9,866	122,660	74,000	2/8/49
A - j	11,200	80,100	59,033	28,933	2/25/49
F - j	3 PPL	3,500	3,033	3 PPL	3/4/49
E - c	7 PPL	6 PPL	5 PPL	5 PPL	3/8/49
G - c	2 PPL	0	1 PPL	1 PPL	3/11/49
H - c	10,200	9,333	12,800	15,733	3/15/49
I - c	7,500	6,000	9,000	8,500	3/17/49
C - c	4,366	25,000	50,800	22,800	3/18/49
J - j	20,066	72,800	89,600	79,333	3/22/49
K - j	26,666	61,866	36,566	28,566	3/24/49
L - c	0	1 PPL	1 PPL	1 PPL	3/25/49
G - c	3 PPL	6 PPL	5 PPL	1 PPL	3/31/49
B - c	7 PPL	12,800	11,200	8,260	4/1/49
M - j	7 PPL	42,133	37,333	14,666	4/6/49
I - c	1 PPL	2 PPL	2 PPL	1 PPL	4/8/49
F - j	1	3,200	3,480	2,666	4/12/49
N - j	36,166	53,666	80,733	61,600	4/14/49
H - j	1 PPL	1 PPL	0	1 PPL	4/19/49
E - c	7 PPL	7 PPL	6 PPL	0	4/22/49
Total	24(161,536	24(511,164	24(637,504	22(364,190	
Average	6,731	21,299	26,563	16,554	
Rating	4	2	1	3	

Key:

* - agar split and fogged
0 - no growth
PPL - Number per plate, not included in average
j - juice canning plant
c - concentrate plant

Media:

1 - Lindegrin's agar
2 - Dextrose tryptone agar
3 - Tryptone glucose extract agar
4 - Sabauroud's agar

Table IV.--Juice to finisher - no. of organisms per ml. of juice

Inoculation - 1 cc. of 1-1000

Plant	Media 1	Media 2	Media 3	Media 4	Date
A - j	18,300	7,800	15,100		1/11/49
B - c	770,000	440,000	550,000		1/14/49
C - c	134,000	116,500	sp.	74,000	1/21/49
D - c	--	--	--	--	2/1/49
E - c	440,000	480,000	440,000	420,000	2/4/49
B - c	78,000	65,000	47,000	74,000	2/8/49
A - j	10 PPL	23,000	27,000	21,000	2/25/49
F - j	39,000	38,000	41,000	28,000	3/4/49
E - c	19,000	21,000	13,000	18,000	3/8/49
G - c	1 PPL	0	1 PPL	1 PPL	3/11/49
H - c	48,000	48,000	46,000	50,000	3/15/49
I - c	3 PPL	2 PPL	1 PPL	2 PPL	3/17/49
C - c	3 PPL	2 PPL	2 PPL	2 PPL	3/18/49
J - j	111,000	35,000	46,000	50,000	3/22/49
K - j	74,000	55,000	70,000	76,000	3/24/49
L - c	--	--	--	--	3/25/49
G - c	118,000	99,000	95,000	111,000	3/31/49
B - c	433,000	450,000	410,000	420,000	4/1/49
M - j	9 PPL	55,000	46,000	21,000	4/6/49
I - c	4 PPL	9 PPL	7 PPL	7 PPL	4/8/49
F - j	61,000	68,000	48,000	97,000	4/12/49
N - j	302,000	365,000	284,000	264,000	4/14/49
H - j	--	--	--	--	4/19/49
E - c	41,000	27,500	sp.	32,500	4/22/49
Total	21(2,687,300	21(2,393,800	21(2,178,100	19(1,756,500	
Average	127,966	113,990	103,719	92,447	
Rating	1	2	3	4	

Key:

j - juice canning plant
c - concentrate plant
-- - not plated
PPL - Number per plate, not included in average
sp. - spreaders

Media:

1 - Lindegrin's agar
2 - Dextrose tryptone agar
3 - Tryptone glucose extract agar
4 - Sabauroud's agar

Table V.--Reconstituted juice or juice from blending tank - no. of organisms per ml. of juice

Inoculation - 1 cc. of 1-1000

Plant	Media				Date
	1	2	3	4	
A - j	44,800	45,840	19,200		1/11/49
B - c	3,000,000	4,666,666	4,200,000		1/14/49
C - c	44,760	28,330	28,090	20,480	1/21/49
D - c	57,140	118,570	74,760	76,190	2/1/49
E - c	500,000	650,000	sp.	71,430	2/4/49
B - c	54,290	91,670	116,666	94,050	2/8/49
A - j	--	--	--	--	2/25/49
F - j	47,000	52,000	50,000	62,000	3/4/49
E - c	120,950	T.N.T.C.	95,240	100,000	3/8/49
G - c	3 PPL	4 PPL	8 PPL	10 PPL	3/11/49
H - c	35,000	27,857	21,904	34,520	3/15/49
I - c	9,760	7,620	4,050	8,330	3/17/49
C - c	17,140	15,480	13,090	15,240	3/18/49
J - j	25,000	33,000	30,000	30,000	3/22/49
K - j	84,000	76,000	60,000	85,000	3/24/49
L - c	21,430	19,420	16,000	22,860	3/25/49
G - c	58,330	29,050	53,090	61,190	3/31/49
B - c	46,910	45,480	67,860	48,810	4/1/49
M - j	25,000	74,000	64,000	44,000	4/6/49
I - c	11,900	12,610	10,710	11,900	4/8/49
F - j	93,000	58,000	38,000	45,000	4/12/49
N - j	44,000	45,000	51,000	47,000	4/14/49
H - j	18,090	9,760	sp.	23,543	4/19/49
E - c	21,670	19,050	sp.	21,430	4/22/49
Total	23(4,330,170	23(6,125,403	23(5,313,660	21(922,973	
Average	118,264	261,974	231,048	43,951	
Rating	3	1	2	4	

Key:

j - canned juice plant
c - concentrate plant
PPL - Number per plate, not included in average
-- - not plated
TNTC - too numerous to count
sp. - spreaders

Media:

1 - Lindegrin's agar
2 - Dextrose tryptone agar
3 - Tryptone glucose extract agar
4 - Sabauroud's agar

Table VI.--Canned concentrate - no. of organisms per ml. of reconstituted product

Inoculation - 1 cc. of 1-1000

Plant	Media 1	2	3	4	Date
B	5,666,666	5,333,333	3,333,333		1/14/49
C	38,570	67,140	57,140	50,000	1/21/49
D	7 PPL	4,280	9 PPL	4,280	2/1/49
E	416,620	400,000	350,000	43,330	2/4/49
B	79,520	84,290	70,950	63,330	2/8/49
E	89,520	34,290	63,330	43,090	3/8/49
G	2,860	3,330	2,380	7 PPL	3/11/49
H	25,470	31,190	21,660	27,620	3/15/49
I	7,860	9,760	6,190	8,090	3/17/49
C	15,940	11,910	13,330	17,860	3/18/49
L	32,290	35,720	34,570	33,710	3/25/49
G	27,140	29,520	25,000	34,290	3/31/49
B	55,238	57,620	54,760	50,480	4/1/49
I	19,760	7,860	7,140	9,050	4/8/49
E	25,000	sp.	sp.	41,660	4/22/49
Total	15(6,502,454	15(6,110,243	15(4,039,683	14(426,590	
Average	433,470	407,349	269,312	30,542	
Rating	1	2	3	4	

Key:

PPL - Number per plate, not included in average
sp. - spreaders

Media:

1 - Lindegrin's agar
2 - Dextrose tryptone agar
3 - Tryptone glucose extract agar
4 - Sabauraud's agar

Table VII.--Fresh juice investigated for pasteurization studies

Inoculation - 1 cc. of 1-1000

Juice	Media					Date
	1	2	3	4	5	
Orange		85,200			198,800	10/8/48
Grapefruit		12,800			9,800	10/25/48
Orange		392,000			672,000	11/8/48
Grapefruit		166,000			225,400	11/15/48
Orange	*	137,000			138,400	11/29/48
Grapefruit	150,000	760,000			400,000	12/6/48
Orange	960,000	1,800,000			1,000,000	12/21/48
Grapefruit	15,540	21,000			10,780	12/28/48
Orange	120,240	108,640			34,080	1/4/49
Orange	89,600	49,920	78,600		44,160	1/7/49
Grapefruit	436,000	425,000	520,000			1/18/49
Grapefruit	980,000	700,000	sp.	1,120,000		1/24/49
Orange	910,000	870,000	1,260,000	840,000		1/31/49
Grapefruit	255,000	200,000	205,000	193,000		2/7/49
Orange	68,000	70,000	66,000	72,000		2/15/49
Orange	1,295,000	630,000	1,420,000	770,000		2/21/49
Grapefruit	136,000	93,000	151,000	156,000		2/28/49
Orange	930,000	880,000	880,000	1,000,000		3/14/49
Grapefruit	93,000	64,000	82,000	84,000		3/21/49
Orange	43,000	74,000	96,000	69,000		3/29/49
Orange	75,000	109,000	109,000	95,000		4/7/49
Grapefruit	147,000	123,000	135,000	164,000		4/11/49
Orange	150,000	88,000	160,000	130,000		4/18/49
Orange	73,000	49,000	58,000	63,000		4/27/49
Orange	1,120,000	700,000	560,000	1,050,000		5/9/49
Orange	1,120,000	800,000	1,260,000	1,600,000		5/16/49
Total	(22)9,166,380	(26)9,407,560	(17)7,600,600	(15)7,406,000	(10)2,733,420	
Average	416,745	361,829	447,094	493,733	273,342	
Total	(15)7,395,000	(15)5,450,000	(15)6,442,000	(15)7,406,000		
Average	493,000	363,333	429,466	493,733		
Rating	2	4	3	1		
Total	(22)9,166,380	(22)8,751,560				
	416,745	397,798				

Key:

* - medium broken and clouded

Media:

1 - Lindegrin's agar
2 - Dextrose tryptone agar
3 - Tryptone glucose extrac agar
4 - Sabauroud's agar
5 - Wort agar

Table VIII.--Rating of media per each medium per each item examined

Items	1	2	3	4	5	6
Lindegrin's agar	2(24)	2(19)	4(24)	1(21)	3(22)	1(15)
Dextrose tryptone agar	1(24)	1(19)	2(24)	2(21)	1(22)	2(15)
Tryptone glucose extract agar	3(24)	4(19)	1(24)	3(21)	2(22)	3(15)
Sabauroud's agar	4(22)	3(17)	3(22)	4(19)	4(20)	4(14)

Key:

Number of tests indicated as
2(24) - medium ranks second. (24) tests were made.

1 - unwashed peel
2 - washed peel
3 - peel at extractors
4 - juice to finisher
5 - juice or reconstituted juice from blending tank
6 - reconstituted juice (canned)

Table IX.--Plate counts comparing buffered and unbuffered juice

Juice	Lindegrin's agar	Dextrose tryptone agar	Wort agar	Date
Orange, unbuffered	*	137,000	138,400	11/29/43
Orange, buffered	*	134,000	166,600	
Grapefruit, unbuffered	750,000	760,000	400,000	12/6/43
Grapefruit, buffered	730,000	770,000	310,000	

* Broken and clouded

Dextrose tryptone agar plus 1 ml. of orange juice per plate - pH 6.0

Dextrose tryptone agar plus 1 ml. of grapefruit juice per plate - pH 5.8

Table X

Lindegrin's agar:

Dextrose	40 grams
KH_2PO_4	1 gram
Peptone	5 grams
Yeast extract	3 grams
Sodium lactate 50%	7 cc.
$MgSO_4$	1 gram
Agar	15 grams
Distilled water	1000 cc.

pH - 5.8

Dextrose tryptone agar:

Agar	15 grams
Tryptone	3 grams
K_2HPO_4	1 gram
Dextrose	1 gram
Distilled water	1000 cc.

pH - 7-7.4

Tryptone glucose extract agar:

Beef extract	3 grams
Tryptone	5 grams
Dextrose	1 gram
Agar	15 grams
K_2HPO_4	1 gram
Distilled water	1000 cc.

pH - 7.0

Sabauroud's dextrose agar:

Peptone	10 grams
Dextrose	40 grams
Agar	15 grams
Distilled water	1000 cc.

pH - 6.2